Identification of a Third Fuzzless Seed Locus in Upland Cotton (*Gossypium hirsutum* L.)

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Segregating populations were developed to evaluate the inheritance of the fuzzless seed phenotypes in upland cotton (*Gossypium hirsutum* L.). Accession 143 of the Mississippi Obsolete Variety Collection (MOVC) has a fuzzless seed phenotype. This line carries the n_2 locus which is recessive to the seed fuzz phenotype. Data from the F_2, BC_1F_1, $F_{2:3}$, and BC_1F_2 populations of DP 5690 × 143 fit a two-loci model for expression of the recessive fuzzless seed phenotype. Fuzzless seeds were obtained in n_2n_2 plants when a second recessive locus (n_3) was present. The dominant N_3 allele found in DP 5690 confers the fuzzy seed phenotype in homozygous n_2 plants. Accession 243 of the MOVC carries the N_1 locus, which is dominant to the presence of seed coat fuzz. No variation from expected ratios was observed in the F_2, BC_1F_1, $F_{2:3}$, and BC_1F_2 populations of the DP 5690 × 243 cross. The N_3 allele had no apparent effect on the expression of the N_1 locus. In a cross between accessions 243 × 143, a few plants were observed which were completely devoid of lint and fuzz fiber (fiberless). A fiberless line was developed from one of these fiberless plants. This line was designated MD 17 fiberless. In a cross between DP 5690 × MD 17 fiberless, we demonstrated that at least three loci were involved in the expression of the fiberless phenotype. The involvement of n_2 and n_3 in the expression of this fiberless phenotype was demonstrated in the F_2 progeny of the cross between 143 × MD 17 fiberless. This is the first demonstration that N_1, n_2, and n_3 interacted to produce fiberless seed.

Cotton (*Gossypium hirsutum* L.) fibers are unicellular trichomes originating from the outer epidermal layer of the seed coat. Fibers are classified into two types, lint and fuzz. The lint fibers initiate growth between anthesis and 2 days postanthesis (DPA) and can elongate 2.5–3.5 cm, whereas the fuzz fibers initiate growth between 5 and 10 DPA and are approximately 0.5 cm (Stewart 1975). During the ginning process the economically important lint fibers are removed from the seed, leaving the much shorter fuzz fiber. Two loci, N_1 and n_2, have been reported that inhibit fuzz fiber development on cottonseeds (for a review see Endrizzi et al. 1985; Percy and Kohel 1999). Either $N_1\_$ or n_2n_2 can produce the ''naked seed'' phenotype. These loci are referred to as the ''naked seed'' alleles and have the phenotype of cottonseeds that produce lint, but they lack fuzz fibers (Endrizzi et al. 1985; Percy and Kohel 1999). To simplify the terminology from the cotton literature, we will refer to ''naked seed'' hereafter as fuzzless seed. This will reduce the confusion to the reader when we introduce the fiberless line, which lacks both fuzz (similar to the fuzzless lines) and lint fibers.

The fuzzless seed phenotype was once believed to be a beneficial trait because of the ease and cleanliness of ginning. However, these lines fell out of favor with geneticists and producers because of their lower lint percentages (Kearney and Harrison 1927; Ware 1940; Ware et al. 1947). With advances in molecular biology, lines with fuzzless and fiberless seed phenotypes have become very important in determining differences in gene/protein regulation during development of ovular trichomes. Joshi et al. (1985, 1988) used histochemical staining to demonstrate reduced activities of β-glycerophosphatase and ATPase in postanthesis epidermal cells of ovules from the fiberless line 9SO × HG. Activities of β-glycerophosphatase and ATPase were easily stained in control cells of the fiber-producing line. The fiberless line,

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designated SL 1-7-1 and listed with the accession number 504 in the Mississippi Obsolete Variety Collection (MOVC; Percival 1987), has also been of great value. This fiberless line has been used to evaluate differences in protein expression between the fiberless and a fiber-producing line during both pre- and postanthesis development (3 days before anthesis to 4 DPA; Turley and Ferguson 1996). Ruan and Chory (1998) also used SL 1-7-1 to demonstrate that, unlike the fiber-producing line, the epithelial cells of SL 1-7-1 had no detectable levels of sucrose synthase protein or its mRNA transcript.

During an attempt to characterize the genotype of the fiberless SL 1-7-1 line, we found aberrations in the simple, one-locus model for the n_2 phenotype. In this article we evaluate the Ballard fuzzless seed line (Kearney and Harrison 1927) which is accession 243 in the MOVC, and the Mexican fuzzless seed UA 3-3, which is accession 143 in the MOVC. Accession 143 has the recessive fuzzless seed allele n_2, which was reported as a simply inherited, one-locus trait (for a review see Endrizzi et al. 1985; Percy and Kohel 1999). Accession 243 has the dominant fuzzless seed allele N_1, which has been genetically linked with a lower lint percentage (Kearney and Harrison 1927; Thadani 1923). A cross between 243 × 143 has allowed the development and release of another fiberless line designated MD 17 fiberless (PI 616493; Turley 2002). In this article we demonstrate a third fuzzless seed locus is required in fuzzless and fiberless seed phenotypes in lines 143 and MD 17 fiberless.

Materials and Methods

Plant Material

Three inbred lines (DP 5690, 143, and 243) along with their resulting F_1, F_2, F_3, BC_1F_1, and BC_1F_2 progeny were grown at Stoneville, MS. DP 5690 was obtained from Delta and Pine Land, Inc. (Scott, MS). Accessions 143 and 243 were obtained from the MOVC managed by Dr. Ed Percival (U.S. National Collection of *Gossypium* Germplasm, Southern Crops Research Laboratory, College Station, TX). The allotments of seed for lines 143 and 243 were grown in the field, verified for phenotype, and seed increased. All field plots were 5 m in length and, where applicable, were overseeded. After the plants reached the first true leaf stage, they were thinned to approximately 6.5 plants/m^2. Weeds and insects were managed using standard agronomic practices for the Mississippi Delta.

We conducted this genetics study from 1996 to 2001 in both the field and glasshouse. The crosses DP 5690 × 143, DP 5690 × 243, and 243 × 143 were made in the field in 1996 and 1998. The minimum size for crossing plots consisted of three rows (1.02 m apart), 5 m in length, with approximately 102 plants. Plants in the crossing plots were sequentially numbered. Plant numbers were recorded on the tags attached to each cross for later verification of the phenotype of the parent plant at harvest. Individual plants of 243, which were used in hybridization, were self-pollinated and the progeny tested to verify homozygosity. All F_1 plants from the crosses were self-fertilized in the glasshouse in the winter of 1997 and 1999, and the F_2 populations were grown in the field in 1997 and 1999. The data from the F_2 populations (1997 and 1999) were combined; however, only plants from 1997 were used to produce the F_2-derived F_3 ($F_{2:3}$) populations. In 1997 two hundred individual F_2 plants from the DP 5690 × 143, DP 5690 × 243, and 243 × 143 crosses were sequentially numbered, harvested, and planted as $F_{2:3}$ populations in a block containing 30 plots in 1997 (plants 1–30 in the F_2 population) and 50 plots in 1999 (plants 31–80 in the F_2 population). We determined that for the segregation of a two-loci model with a 99% confidence level, we would need to evaluate 72 $F_{2:3}$ populations. An additional eight $F_{2:3}$ populations were evaluated, which increased the total populations to 80, as listed above.

The F_1 plants from the DP 5690 × 143, DP 5690 × 243, and 243 × 143 crosses grown in 1999 were either self-fertilized or used for backcrosses with DP 5690, 143, and 243. The BC_1F_1 populations were grown in the field in 2000 and BC_1F_2 populations were grown in the field in 2001. During the course of this project, the MD 17 fiberless line was produced from an individual selection of the 243 × 143 cross and released as a genetic stock (Turley 2002). Crosses of DP 5690 × MD 17 fiberless, 143 × MD 17 fiberless, and 243 × MD 17 fiberless were made to verify the genotype of the fiberless phenotype in 2000. These F_1 plants were self-fertilized or backcrossed in the glasshouse in the winter of 2001 and then planted in the field in the spring of 2001. The genetic models tested for each population are listed in the Results. Chi squares were calculated to determine the best fit for all genetic models tested.

The fuzzy/fuzzless phenotypes were scored as described by Ware et al. (Ware 1940; Ware et al. 1947), with the fuzzy seed corresponding to classes 1–9 and fuzzless seed corresponding to classes 13–19. These groupings of fuzzy and fuzzless seed were originally used by Ware et al. (1947) during the discovery of the recessive fuzzless seed phenotype. The fiberless phenotype corresponded to class 20 (Ware 1940). Determination of plant phenotype was assessed by examining seeds from open capsules at the first branch node between main stem nodes 7–10.

Natural outcrossing of cotton was measured in the field with the use of three phenotypic markers: virescent leaf, red leaf, and glandless (Xanthopoulos and Kechagia 2000). Single-row plots of these marker plants were randomly placed throughout the field in 1996–2000. We used both individual plant harvest and bulk collections to obtain seed for planting the following year. Outcrossing from the previous year was evaluated by overseeding individual plots randomly placed throughout the field. These plants were grown to the stage of two to four fully expanded leaves, at which time the leaf color or gland pattern was visible. The number of outcrosses and total plants were counted and the percentage was determined to be 0.83% in 1997, 1.5% in 1998, 1.3% in 1999, and 1.5% in 2000. The outcrosses in each plot were removed and then plants were thinned to approximately 6.5 plants/m^2.

Results

A summary of the expected and proposed genotypes of DP 5690, 143, 243, and MD 17 fiberless, along with their respective lint percentages and phenotypes, are listed in Table 1. Using the lint percentage of DP 5690 as the standard (100%), a trend of decreasing lint percentage is reported for lines carrying the fuzzless seed genotypes, that is, lines 143 (63%), 243 (28%), and MD 17 fiberless (0%). This decrease in lint percentage of the parental lines DP 5690, 243, 143, and MD 17 fiberless can be visualized in Figure 1. Both Table 1 and Figure 1 list a newly proposed locus as a recessive, fuzzless seed allele, which we have designated as n_3. The n_3 locus is required

for expression of the fuzzless and fiberless phenotypes in lines 143 and MD 17 fiberless, respectively. Parental lines DP 5690, 143, 243, and MD 17 fiberless were homozygous for the three loci listed in Table 1 (Table 2). Abbreviations for fuzzy seed coat (F), fuzzless seed coat (N), and fiberless (fls) are used in Table 2 and in ratios reported in the text to facilitate the description of segregation patterns.

The cross of DP 5690 × 143 produced F_1 and BC_1F_1 (F_1 × DP 5690) populations in which all plants had seed fuzz, indicating that line 143 was recessive for the fuzzless seed trait (Table 2). The F_2 and BC_1F_1 (F_1 × 143) populations had segregation patterns (Table 2) that did not fit a simple one-locus inheritance model ($\chi^2_{3:1,1}$ df = 56.77, P = .00000), as was reported in the literature for this variant (Endrizzi et al. 1985; Percy and Kohel 1999; Ware et al. 1947). The F_2 data are the combined progeny numbers from 2 years of crosses between DP 5690 × 143 in 1996 and 1998. Data fits a two loci model with a 15(F):1(N) ratio and $\chi^2_{15:1,1}$ df = 0.0213, P = .88387 (200 progeny in 1997) and a $\chi^2_{15:1,1}$ df = 1.2298, P = .26745 (141 progeny in 1999). The BC_1F_1 (F_1 × 143) segregated in a 3(F):1(N) ratio, also confirming the existence of a second recessive locus in line 143. Furthermore, results from the $F_{2:3}$ (total of 1573 progeny) and BC_1F_2 (total of 1416 progeny) populations strongly indicated that two independent loci were involved in the expression of the recessive fuzzless seed phenotype, as shown in Table 3. We designated this second locus as n_3. In its recessive form, n_3 is required for the expression of the recessive fuzzless seed allele n_2 in the DP 5690 × 143 cross. The N_3_ genotype prevents the expression of the fuzzless seed phenotype in the homozygous n_2 plant. The normal cultivar, DP 5690, would therefore have the genotype of $n_1n_1N_2N_2N_3N_3$, whereas 143 would have the genotype $n_1n_1n_2n_2n_3n_3$ (Table 1). The data indicate that n_2 and n_3 are not closely linked.

A list of the models tested for the F_2 and BC_1 (F_1 × 143) populations of the DP 5690 × 143 cross is listed in Table 4. These models range from the single-locus model to variations of a three-loci model. The third loci was labeled as n_4 in Table 4 and evaluated as both a dominant and recessive allele which influenced either n_2 or n_3 directly. An additional model, not included in Table 4, assumes that the fuzzless phenotype may be the result of the $n_2n_2N_4$_ genotype (genotype of DP 5690 would then be $N_2N_2N_3N_3n_4n_4$). With this model the F_2 data result in a $\chi^2_{13:3,1}$ df = 29.1848, P = .00000, and the BC_1 (F_1 × 143) data result in a $\chi^2_{1:1,1}$ df = 10.5932, P = .00114.

Only one manifestation of outcrossing was observed in the entire experiment. Because outcrossing for the 4 years averaged 1.28% (Materials and Methods), deviations from ratios in most populations would not be statistically detected. However, for populations which were expected to be either 100% fuzzy seed

Table 1. A summary of expected and proposed genotypes with lint percentages from four lines: DP 5690, 143, 243, and MD 17

	Genotype				
Variety	Expected	Proposed	Lint %[a]	References for lint %	Phenotype
DP 5690	$n_1n_1N_2N_2$	$n_1n_1N_2N_2N_3N_3$	40.5	NCVT[b] 1995	Normal
143	$n_1n_1n_2n_2$	$n_1n_1n_2n_2n_3n_3$	25.6	NGRP[c] 2001a	Fuzzless
243	$N_1N_1N_2N_2$	$N_1N_1N_2N_2n_3n_3$	11.4	NGRP[c] 2001b	Fuzzless
MD 17	$N_1N_1n_2n_2$	$N_1N_1n_2n_2n_3n_3$	0.00	Turley 2002	Fiberless

[a] Lint percentage is calculated as lint weight divided by total weight (lint and cottonseed) multiplied by 100.
[b] NCVT, National Cotton Variety Trial (see References).
[c] NGRP, National Genetics Resources Program (see References).

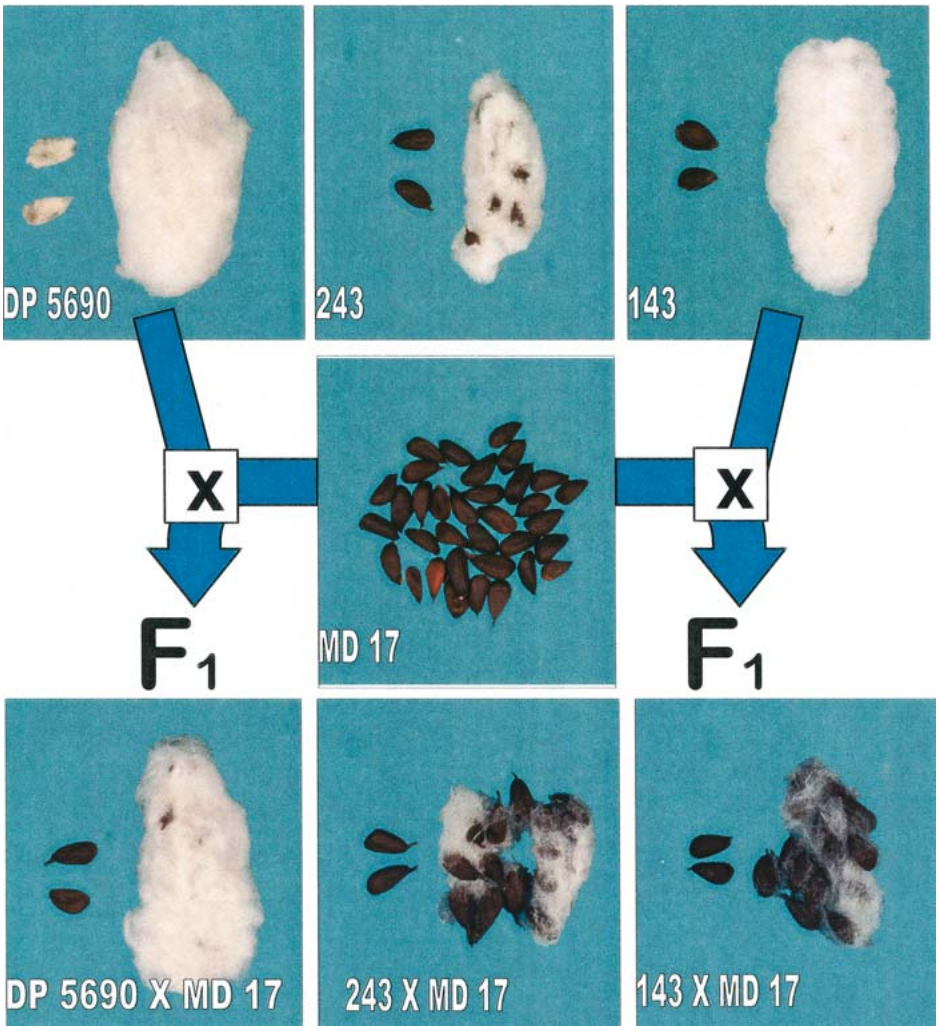

Figure 1. Photographs of two delinted seeds and a representative locule from a cotton capsule. DP 5690 with the proposed genotype $n_1n_1N_2N_2N_3N_3$ (normal phenotype). 243 with the proposed genotype $N_1N_1N_2N_2n_3n_3$ (fuzzless seed phenotype). 143 with the proposed genotype $n_1n_1n_2n_2n_3n_3$ (fuzzless seed phenotype). MD 17 fiberless with the proposed genotype $N_1N_1n_2n_2n_3n_3$ (fiberless seed phenotype). DP 5690 × MD 17, the F_1 progeny of the cross with the proposed genotype $N_1n_1N_2n_2N_3n_3$ (fuzzless seed phenotype). MD 17 × 243, the F_1 progeny of the cross with the proposed genotype $N_1N_1N_2n_2N_3n_3$ (fuzzless seed phenotype). MD 17 × 143, the F_1 progeny of the cross with the proposed genotype $N_1n_1n_2n_2n_3n_3$ (fuzzless seed phenotype).

Table 2. Summary of crosses and generations of DP 5690, 143, 243, and MD 17

Cross and generation	Observed number of plants			Number of segregating loci	Expected ratio	χ^2
	F	N	fls			
DP 5690	168	—	—			
143	—	140	—			
243	—	108	—			
MD 17	—	—	184			
DP 5690 × 143						
F_1	48	—	—	2	1F:0N[a]	—
BC_1 (F_1 × 143)	42	17	—	2	3F:1N	0.4573
BC_1 (F_1 × DP 5690)	104	—	—	2	1F:0N	—
F_2[b]	316	25		2	15F:1N	0.6805
DP 5690 × 243						
F_1	—	48	—	2	0:1N	—
BC_1 (F_1 × 243)	—	180	—	2	0:1N	—
BC_1 (F_1 × DP 5690)	111	110	—	2	1F:1N	0.0045
F_2[b]	80	280	—	2	1F:3N	1.4815
243 × 143						
F_1	—	84	—	2	0F:1N	—
BC_1 (F_1 × 143)	41	99		2	1F:3N	1.3714
BC_1 (F_1 × 243)	—	106	—	2	0F:1N	—
F_2[b]	64	287	23	2	3F:12N:1fls	0.6916
MD 17 × DP 5690						
F_1	—	31	—	3	—	—
F_2	41	141	2	3	15F:48N:1fls	0.4362
MD 17 × 143						
F_1	—	36	—	1	1N:0fls	—
F_2	—	113	30	1	3N:1fls	0.2668

[a] F, fuzz-covered seed; N, fuzzless seed; fls, fiberless.

[b] Population totals are the sum of plants from multiple crossings and plantings in different years.

or fuzzless seed, minor deviations become very noticeable. Our two-loci model predicts either a 15(F):1(N), 3(F):1(N), or 0(F):1(N) for $F_{2:3}$ population of the DP 5690 × 143 cross. We observed a deviation from a population derived from a fuzzless F_2 plant which should have segregated in a 0(F):1(N) ratio. The actual segregation was 4(F):37(N). We believe this deviation to be an outcross by a dominant N_2 plant.

We, as did Kearney and Harrison (1927), used line 243 as the source of the N_1 allele. The cross DP 5690 × 243 was made to confirm the genetics of accession 243 with a modern cotton cultivar. Our data support the single-gene model (N_1) as was initially reported (Kearney and Harrison 1927). The observed effects of the dominant fuzzless seed locus on the reduction of lint percentage and fiber quality in the progeny of the DP 5690 × 243 cross appeared to be indistinguishable from descriptions in earlier reports (Kearney and Harrison 1927; Thadani 1923; Ware 1940).

Having replicated Kearney and Harrison's (1927) results with lines 243 and DP 5690, we crossed line 243 with 143 to evaluate the interaction of the N_1, n_2, and n_3 loci. The F_1 population was completely fuzzless seed, as was expected; however, the BC_1F_1 (F_1 × 143) and F_2 data fit a two-loci model ($\chi^2_{3:12:1,1}$ df = 0.6916, P = .70765; Table 2). The expected three-loci model for the F_2 population of the 243 × 143 cross did not fit the data with a predicted ratio of 15(F):48(N):1(fls) and a $\chi^2_{15:48:1,2}$ df = 56.9027, P = .00000. Therefore we conclude either that lines 143 and 243 share a common homozygous locus, n_3, or that n_3 is not required for the expression of n_2, when n_2 is associated with its homologous counterpart N_1 ($N_1N_1n_2n_2$; Samora et al. 1994). Data collected from other crosses in this manuscript (DP 5690 × MD 17) support the common-locus model. With a shared locus, the two-loci model can be easily explained.

Individual plants were collected from the BC_1F_1 (F_1 × 143) population, with 12 plants selected to be evaluated as BC_1F_2 populations. Six BC_1F_1 plants with fuzzy seed (deduced genotype $n_1n_1N_2n_2n_3n_3$) were selected and the resulting BC_1F_2 populations all fit a 3(F):1(N) ratio and included a total of 252(F):85(N) plants with a $\chi^2_{3:1,1}$ df = 0.0089, P = .92483. Six BC_1F_1 plants with fuzzless seed (deduced genotypes $N_1n_1N_2n_2n_3n_3$, $N_1n_1n_2n_2n_3n_3$, and $n_1n_1n_2n_2n_3n_3$) were difficult to evaluate due to smaller population sizes. Five of the six populations fit into either a 1(N):0(F) (three populations) or a 3(N):1(fls) (two populations) ratio. The sixth population had some fuzzy seed plants, and therefore the expected ratio in our model would be 3(F):12(N):1(fls). However, no fiberless plants were observed in the sixth population (33 plants). The results of BC_1F_2 populations also fit the two-loci model.

The characterization of line 243 as homozygous for the n_3 locus allows us to deduce the effects of a dominant N_3 allele on the phenotypic expression of N_1. In a review of the DP 5690 × 243 cross, the F_2 progeny segregated 80(F):280(N) (Table 2). If N_3_ reversed the expression of N_1, as it did for n_2, the segregation of the F_2 progeny would have been reversed with 13(F):3(N), $\chi^2_{3:1,1}$ df = 823.36, P = .00000. A second model was evaluated with only homozygous N_3N_3 reversing the expression of the fuzzless seed allele N_1_. The segregation ratio for this scenario would be 6(F):9(N) with a $\chi^2_{6:9,1}$ df = 47.4074, P = .00000. Therefore N_3 had no noticeable effects on the expression of the N_1 fuzzless seed phenotype. However, we do not know whether N_3 has an effect on the expression of lint percentage.

Twenty-three of the 374 F_2 progeny (243 × 143) were fiberless (Table 2). These fiberless plants were of great

Table 3. Segregation patterns of progeny rows from the $F_{2:3}$, BC_1F_2 (F_1 × 143), and BC_1F_2 (F_1 × DP 5690) populations of the DP 5690 × 143 cross

Cross and generation	No. of progeny rows				Model of two loci	
	All F[a]	15F:1N	3F:1N	All N	Expected ratio	χ^2
DP 5690 × 143						
$F_{2:3}$ from fuzzy seed	32	17	27	—	7:4:4	3.1024
$F_{2:3}$ from fuzzless seed	—	—	—	4	—	—
BC_1F_2 (F_1 × 143) from fuzzy BC_1F_1		4	4	—	1:2[b]	1.0000
BC_1F_2 (F_1 × 143) from fuzzless BC_1F_1				2	—	—
BC_1F_2 (F_1 × DP 5690) from fuzzy BC_1F_1	8	2			3:1[c]	0.1333

[a] F, fuzz-covered seed; N, fuzzless seed; fls, fiberless.

[b] Segregation ratio should be 1(15F:1N):2(3F:1N):1(1N:0F).

[c] Segregation ratio should be 3F to 1 line which segregates 15F:1N.

interest because they could be used as a genetic tool to evaluate fuzzless and fiberless lines, such as SL 1-7-1 (Turley and Ferguson 1996). In the 30 F_2 (243 × 143) plants grown in 1997, only one plant, and its resulting $F_{2:3}$ population, was fiberless. A single F_3 plant was selected and developed into a genetic stock called MD 17 fiberless (Turley 2002).

A cross between DP 5690 × MD 17 fiberless was made and the F_1 data are shown in Table 2. The F_1 progeny were all fuzzless (31 plants), indicating that MD 17 fiberless was homozygous for the N_1 allele (Figure 1). We used a 143 × MD 17 fiberless cross to demonstrate the involvement of both recessive fuzzless seed alleles n_2 and n_3 in producing the fiberless phenotype of MD 17 fiberless. All 144 plants in the F_2 progeny had fuzzless seed, an indication that MD 17 fiberless was homozygous for both n_2 and n_3 alleles. Any deviation from homozygosity ($n_2n_2n_3n_3$) in MD 17 fiberless would have resulted in segregating populations with fuzzy seed progeny. Therefore we report the genotype for MD 17 fiberless to be $N_1N_1n_2n_2n_3n_3$. This genotype is modified from the original report of MD 17 fiberless (Turley 2002) in that the homozygous n_3 allele is now included.

Representative F_1 progeny from the crosses of DP 5690 × MD 17 fiberless, 143 × MD 17 fiberless, and 243 × MD 17 fiberless are shown in Figure 1. The reduction in lint on these F_1 seeds is easily visualized. Similar lint reductions are observed whether these progeny are grown in the glasshouse or the field. The interaction of these loci appears to have a reductive effect on lint production. The N_1_ locus is dominant for fuzzless seed; however, homozygous N_1N_1 is required for the expression of the fiberless phenotypes. Both the N_1n_1 genotype in combination with the homozygous $n_2n_2n_3n_3$ ($N_1n_1n_2n_2n_3n_3$) or the N_2n_2 genotype in combination with $N_1N_1n_3n_3$ ($N_1N_1N_2n_2n_3n_3$) produce plants with seeds that are sparsely linted (Figure 1; 143 × MD 17 and 243 × MD 17).

With information obtained from the crosses and observations as described above, we report the test of two possible models for determination of the fiberless phenotype. The three-loci model would segregate in a 15(F):48(N):1(fls) ratio. The actual data fit this model with a $\chi^2_{15:48:1,2}$ df = 1.1629, P = .55909. The two-loci model was also tested assuming homozygous N_1 and n_2 would give a fiberless plant. The expected ratio was 3(F):12(N):1(fls) and was evaluated to have a $\chi^2_{3:12:1,2}$ df = 9.1377, P = .01037. Other loci may also be involved in the expression of the fiberless phenotype, but with the small size of the F_2 population (only 184 plants), the interaction of a fourth or fifth allele at this point would be difficult to verify or eliminate.

In the F_2 progeny of 143 × MD 17 fiberless we observed 30 fiberless plants, but seven other plants produced seed which was sparsely linted (similar to Figure 1; 143 × MD 17). These seven plants may carry an unknown locus (or loci) that is influenced by specific environmental conditions and slightly modifies the expression of the fiberless trait. Using the segregation ratio of 113(N):30(fls) would give a $\chi^2_{3:1,1}$ df = 1.2331, P = .26680 (Table 2).

Table 4. Proposed and alternate genotypes, expected segregation ratios, and chi squares for F_2 and BC_1 (F_1 × 143) populations of the cross DP 5690[a] × 143

Genotype tested for fuzzless phenotype	F_2 segregation ratio	χ^2	BC_1 (F_1 × 143) segregation ratio	χ^2
n_2n_2	3F:1N	56.78***	1F:1N	10.59**
$n_2n_2n_3n_3$	15F:1N	0.6805	3F:1N	0.4573
$n_2n_2n_3n_3n_4n_4$[b]	57F:7N	4.552*	5F:3N	1.899
$n_2n_2n_3n_3n_4n_4$[c]	55F:9N	12.78***	1F:1N	10.59**
$n_2n_2n_3n_3n_4n_4$[d]	61F:3N	5.335*	7F:1N	14.36***
$n_2n_2n_3n_3n_4n_4$[e]	63F:1N	73.78***	7F:1N	14.36***

[a] DP 5690 is proposed to be $N_2N_2(N_3N_3)$ (N_4N_4) for all crosses.
[b] Fuzzless phenotype conferred by either $n_2n_2n_3n_3$ or $n_2n_2n_4n_4$.
[c] Fuzzless phenotype conferred by either $n_2n_2n_3n_3$, $n_2n_2n_4n_4$, or $n_3n_3n_4n_4$.
[d] Fuzzless phenotype conferred by $n_2n_2n_3n_3N_4$_.
[e] Fuzzless phenotype conferred by $n_2n_2n_3n_3n_4n_4$.
*P < .05; **P < .01; ***P < .001.

Discussion

The ratios from the F_2, $F_{2:3}$, BC_1F_1, and BC_1F_2 of the DP 5690 × 143 cross in Tables 2 and 3 indicated that two recessive loci (n_2 and n_3) were required for the fuzzless seed phenotype. We have deduced that the fuzzless seed loci, n_2 and n_3, are involved in expression of the fiberless trait of MD 17 fiberless. The F_2 population of the 143 × MD 17 fiberless was 100% fuzzless seed. For this to occur, MD 17 would have to be homozygous for the n_2 and n_3 genotypes. Also the F_2 progeny of the 143 × MD 17 fiberless cross fit the expected 3(N):1(fls) model. Therefore the three alleles responsible for the phenotype of MD 17 fiberless were N_1, n_2, and n_3.

Ware et al. (1947) first reported the recessive fuzzless seed locus in cotton. This observation was based solely on F_1 populations of crosses between Acadian brown (n_2) and eight different fuzzy seed varieties. All eight crosses produced F_1 progeny that were fuzzy seeded. The allele was designated n_2 by Kohel (1973) and mapped to chromosome 26 (Endrizzi et al. 1985; Samora et al. 1994). The control of the recessive fuzzless seed phenotype by a single locus may be more common in crosses between n_2n_2 and obsolete cotton lines. Both obsolete lines, 143 and 243, used in this study, and the fiberless line SL 1-7-1 (Turley RB and Kloth RH, unpublished data) were homozygous for n_3n_3.

In our results we emphasized the discovery of the recessive form of the fuzzless seed allele n_3 because it is required for both fuzzless and fiberless phenotypes. In actuality, the important allele is the dominant N_3, which limits the expression of the recessive fuzzless seed phenotype and its associated decrease in lint percentage. Therefore, in combination with the recessive n_1 and the dominant N_2 (independently these loci express the fuzzy seed phenotype), we have three loci that influence seed fuzz. Elucidation of the genes/proteins responsible for the expression of these alleles would allow us to develop strategies to increase the percentage of epidermal cells that initiate into fiber and to improve lint quality.

The epistatic interaction of the fuzzless seed alleles to produce a fiberless seed is also of great interest. Ware et al. (1947) originally reported finding fiberless lines in the F_2 population of a cross between Acadian Brown (n_2n_2) × Acala Mex (N_1N_1). The importance of these fiberless plants was basically ignored, as they were counted as fuzzless seed observations in these data. Ware et al. (1947) reported an F_2 population of 8(F):38(N):3(fls) which fit a two-loci model with a $\chi^2_{3:12:1,2}$ df = 0.1973, P =

.90607. Therefore we hypothesize that Acadian Brown and Acala Mex also possess the recessive fuzzless seed allele n_3.

We can distinguish at least three genetic mechanisms that produce the fiberless phenotype. The MD 17 fiberless line has been described above in great detail. One reason for the development of the MD 17 fiberless line was for use in genetic proofs of other fiberless lines, such as SL 1-7-1 (Turley and Ferguson 1996). Both MD 17 and SL 1-7-1 share two common loci, the fuzzless seed alleles N_1 and n_3 (Turley RB and Kloth RH, unpublished data). The third genetic mechanisms to produce a fiberless line is reported for MCU.5.

MCU.5 fiberless was first reported by Peter et al. (1984) to be a natural mutant of MCU.5 (*G. hirsutum* L.). Nandarajan and Rangasamy (1988) studied the inheritance pattern for the fiberless line by crossing it with five different *G. hirsutum* lines. No reference was made as to the phenotype of the F_1 progeny of these crosses. However, an evaluation of the F_2 progeny indicated segregation ratios of 15(F):1(fls), 63(F):1(fls), and 255(F):1(fls) (Nandarajan and Rangasamy 1988). Unlike our study, no fuzzless seed lines were observed. Nandarajan and Rangasamy (1988) concluded that two to four gene pairs are responsible for the recessive fiberless trait.

The absence of the fuzzless seed phenotype in the data from Nandarajan and Rangasamy (1988) was a major deviation from our results and other reports on the genetics of the fuzzless seed alleles (Kearney and Harrison 1927; Thadani 1923; Ware et al. 1947). The initial conclusions indicate the absence of at least the dominant fuzzless seed allele N_1 from MCU.5 fiberless. Presently no locus has been reported in the literature to inhibit the expression of N_1. We report herein that N_3 had no visible effect on the expression of the fuzzless seed phenotype by N_1.

Numerous loci have yet to be identified which modify the expression of lint development in *G. hirsutum* L. Endrizzi and Ramsey (for a review see 1979) reported three monosomic lines that differed in seed fuzz expression. These were monosome 17 (less seed fuzz) and monosomes 18 and 20 (dense seed fuzz). All three monosomes were found in the D subgenome of allotetraploid cotton and were not linked to the N_1 or n_2 alleles. The fuzzless seed alleles N_1 and n_2 have been mapped to homologous chromosomes 12 and 26 (for a review see Endrizzi et al. 1985; Percy and Kohel 1999; Samora et al. 1994). With at least five chromosomes—12, 17, 18, 20, and 26-carrying loci that influence fuzz development, the complex nature of fuzz development becomes very apparent. Interaction of these loci, if any, remains a mystery.

An effort is now being made to identify all the alleles responsible for the fuzzless seed phenotype. The fuzzless seed phenotype allows us to visually follow these alleles through different crosses, facilitating a correlation with gene/protein expression. The identification of these alleles may also give us insights into the biology of fiber initiation, better fiber quality, and increasing lint percentage. Lint percentage is a major component of fiber yield. In recent years cotton yields have reached a plateau, stagnating the growth and development of the cotton industry in the United States. Any improvements in lint percentage could theoretically increase yields of cotton fiber production.

References

Endrizzi JE and Ramsey G, 1979. Monosomes and telosomes for 18 of the 26 chromosomes of *Gossypium hirsutum.* Can J Genet Cytol 21:531–536.

Endrizzi JE, Turcotte EL, and Kohel RJ, 1985. Qualitative genetics, cytology, and cytogenetics. In: Cotton (Kohel RJ and Lewis CF, eds). Madison, WI: Agronomy Society of America.

Joshi PA, Stewart JM, and Graham ET, 1985. Localization of β-glycerophosphatase activity in cotton fiber during differentiation. Protoplasma 125:75–85.

Joshi PA, Stewart JM, and Graham ET, 1988. Ultrastructural localization of ATPase activity in cotton fiber during elongation. Protoplasma 143:1–10.

Kearney TH and Harrison RJ, 1927. Inheritance of smooth seed in cotton. J Agric Res 35:193–217.

Kohel RJ, 1973. Genetic nomenclature in cotton. J Hered 64:291–295.

Nadarajan N and Rangasamy SR, 1988. Inheritance of the fuzzless-lintless character in cotton (*Gossypium hirsutum*). Theor Appl Genet 75:728–730.

National Cotton Variety Trial, 1995. National Cotton Variety Trial. Accessed November 16, 2001, at http://msa.ars.usda.gov/ms/stoneville/cgrp/ncvt/95/1995book.htm.

National Genetic Resources Program, 2001a. National Plant Germplasm System. PI 528543. Accessed November 16, 2001, at http://www.ars-grin.gov/cgi-bin/npgs/html/obs.pl?1423479.

National Genetic Resources Program, 2001b. National Plant Germplasm System. PI 528610. Accessed November 16, 2001, at http://www.ars-grin.gov/cgi-bin/npgs/html/obs.pl?1423546.

Percival AE 1987. The national collection of *Gossypium* germplasm. Southern Cooperative Series Bulletin 321.

Percy RG and Kohel RJ, 1999. Qualitative genetics. In: Cotton: origin, history, technology, and production (Smith CW and Cothren JT, eds). New York: John Wiley & Sons; 319–360.

Peter SD, Muralidharan V, and Vaman Bhat M, 1984. Fuzzless-lintless mutant of MCU 5 cotton. Cotton Dev 14:43.

Ruan Y-L and Choury PS, 1998. A fiberless seed mutation in cotton is associated with lack of fiber cell initiation in ovule epidermis and alterations in sucrose synthase expression and carbon partitioning in developing seeds. Plant Physiol 118:399–406.

Samora PJ, Stelly DM, and Kohel RJ, 1994. Localization and mapping of Le_1 and Gl_2 loci of cotton (*Gossypium hirsutum*). J Hered 85:152–157.

Stewart JM, 1975. Fiber initiation on the cotton ovule (*Gossypium hirsutum*). Am J Bot 62:723–730.

Thadani KI, 1923. Linkage relationships in the cotton plant. Agric J India 18:37–42.

Turley RB, 2002. Registration of MD 17 fiberless upland cotton as a genetic stock. Crop Sci 42:994–995.

Turley RB and Ferguson DL, 1996. Changes in ovule proteins during early fiber development in a normal and a fiberless line of cotton (*Gossypium hirsutum* L.) J Plant Physiol 149:695–702.

Ware JO, 1940. Relation of fuzz pattern to lint in an upland cotton cross. J Hered 31:489–498.

Ware JO, Benedict LI, and Rolfe WH, 1947. A recessive naked-seed character in upland cotton. J Hered 38:313–320.

Xanthopoulos FP and Kechagia UE, 2000. Natural crossing in cotton (*Gossypium hirsutum* L.). Aust J Agric Res 51:979–983.

Received January 11, 2002
Accepted August 8, 2002

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